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Competition between two planktonic rotifer species at different temperatures: an experimental test

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SUMMARY

1. The effect of temperature on the outcome of resource competition between two planktonic rotifers (*Synchaeta pectinata* and *Brachionus calyciflorus*) was investigated in laboratory experiments. In addition to the competition experiments, several physiological variables and their temperature-dependence were characterised, including ingestion rate and starvation tolerance.
2. Because of a lower threshold food level (TFL) for population growth for the food algae *Cryptomonas erosa*, *Synchaeta* was predicted to be the superior competitor at low temperatures (12 °C). In contrast, *Brachionus* had a lower TFL at 20 °C and was predicted to be competitively superior at this temperature.
3. In both rotifer species, ingestion rates increased with temperature, but the increase was much more pronounced in *Brachionus*. Ingestion rates of *Brachionus* at temperatures from 8 to 24 °C were always higher than in *Synchaeta* (up to 4.6-fold).
4. Starvation resistance reduced with temperature in both rotifer species. At all temperatures investigated (12, 16 and 20 °C) *Brachionus* could survive starvation for longer than *Synchaeta*. This difference was strongest at 12 °C (5.8 days versus 2.5 days).
5. In the first competition experiment, food was supplied at 48 h-intervals. *Brachionus* displaced *Synchaeta* at both experimental temperatures (12 and 20 °C). Competitive exclusion of *Synchaeta* at the lower temperature was probably because of large fluctuations in algal densities that resulted from the long intervals between feeding, a condition that favoured *Brachionus* because of its higher starvation resistance.
6. In the second competition experiment, one third of the food suspension was renewed every 8 h, resulting in a much better approximation to a continuous resource supply. At 12 °C *Synchaeta* and *Brachionus* coexisted for more than 1 month and the densities of both rotifer species were significantly lower in the presence of their competitor. In contrast to expectations, *Brachionus* was able to persist even when *Cryptomonas* concentrations fell below its TFL. This was probably because *Brachionus* was using detritus and associated bacteria as additional food sources, which were present in the cultures during the later phase of the experiment.
7. Autocorrelation analysis of the temporal changes in egg ratios revealed significant periodic cycles in *Synchaeta* during the second competition experiment. A possible explanation for this is the fecundity schedule of *Synchaeta*, in which reproduction is highly concentrated in a few age classes. According to demographic theory, such a life cycle feature can cause slower convergence to a stable age distribution.

Keywords: microcosm, population growth, resource competition, Rotifera, temperature change

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Introduction

Ecologists have long tried to understand the distribution of species in relation to temperature clines. The importance of understanding such patterns has renewed emphasis because of research on the effects of global warming (Hughes, 2000; Stenseth *et al.*, 2002). One formerly popular approach to predict changes in the composition of species was the 'climate-mapping approach', which consists of mapping the distribution of a species in climate-space and then, if the position of that climate-space changes, predicting the shift of the species' distribution. This approach, as well as other simplistic measures of the temperature preference of a species (e.g. abundances, physiological tolerance, or growth performance in relation to temperature), are however inadequate for predictions at the community level, because biological processes like predation, competition or dispersal can have significant influences on patterns of species abundances (Davies *et al.*, 1998a,b; Jiang & Morin, 2004). In addition, some of these processes are temperature-dependent themselves; for instance, competition coefficients may change along temperature gradients. Thus a deeper understanding of the processes affecting the distribution of species in relation to temperature may require a characterisation of the actual mechanisms underlying such temperature-related changes.

Resource competition theory (Tilman, 1982) has been very successful in predicting the outcome of competition using a mechanistic approach. Rather than lumping competitive effects into the competition coefficient, the theory predicts the outcome of competition between two or more species on the basis of individual resource requirements. The simple prediction in the case of *n* competitors and one resource is that the species with the lowest food requirement to maintain zero population growth (threshold food level, TFL) will always win. Resource competition theory can also be extended to more complex situations, such as where more than one resource type is available. Its successful application has been demonstrated for several organisms, including algae and mesozooplankton (Tilman, 1982; Rothhaupt, 1988; Kreutzer & Lampert, 1999).

Threshold food levels are not species-specific constants but can vary depending on several conditions. For example Rothhaupt (1990a) found that the TFLs of two *Brachionus* species depend on the size of their food algae. The larger *Brachionus calyciflorus* Pallas showed the lowest TFL when it was cultured with the relatively large algae *Chlamydomonas sphaeroides* Gerloff, whereas the smaller *Brachionus rubens* Ehrenberg had the lowest TFL with the relatively small algae *Monorhaphidium minutum* Nägeli (Rothhaupt, 1990a). Rothhaupt (1990b) suggested that this was because of the differential efficiency of the two rotifers to ingest particles of different sizes and this, in turn, seemed to depend on the relationship between the size of the rotifer and of its food algae. Food algae-dependent TFLs were also found by Ciros-Perez, Carmona & Serra (2001) for three species of the *Brachionus plicatilis* complex, although in this study no clear relationship with animal size was apparent.

Temperature is another environmental factor that can modify a species' TFL (Achenbach & Lampert, 1997; Stelzer, 1998; Weisse *et al.*, 2002). In most cases TFLs stay constant for a range of temperatures and increase at higher temperatures. In some cases the TFL may also be elevated at very low temperatures, leading to a U-shaped pattern of TFL in relation to temperature (e.g. Weisse *et al.*, 2002). An application of resource competition theory to species distributions along temperature clines involves measurements of the TLFs of the different species at different temperatures. If the TFL-temperature isoclines of two species run parallel, no shifts in competitive dominance are expected. Such a result was obtained by Achenbach & Lampert (1997) for four differentially sized Daphnia species. However, if such TFL-temperature isoclines do cross, resource competition theory predicts that one species will be superior at low temperatures while the other will be superior at high temperatures. Such a situation was described by Tilman, Mattson & Langer (1981) for two diatom species. The authors found that *Asterionella formosa* Hassal competitively displaced *Synedra ulna* Ehrenberg at temperatures below 20 °C but that the situation was reversed at temperatures above 20 °C. This outcome was predicted by measurements on the silicate growth kinetics of the two algae at different temperatures (Tilman *et al.*, 1981). In the present paper I examine an analogous situation in two heterotrophic consumers.

Laboratory experiments and field data suggest that the two rotifers *Synchaeta pectinata* Ehrenberg and *B. calyciflorus* have different thermal preferences. In a study involving several hundred freshwater habitats,

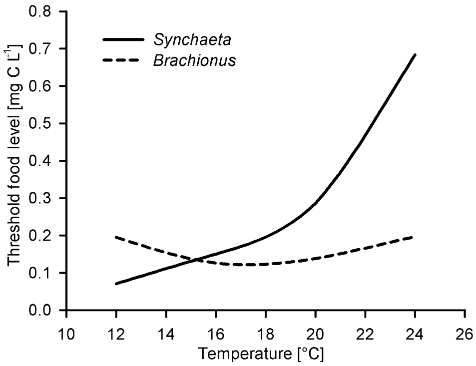

Fig. 1 Temperature-dependence of the threshold food levels of *Synchaeta pectinata* and *Brachionus calyciflorus* feeding on *Cryptomonas erosa*. Redrawn from Stelzer (1998).

sampled during different years and different seasons, Bērzinš & Pejler (1989) established frequency distributions of 225 rotifer species in relation to water temperature. The highest abundances of *Synchaeta* were found at 12 °C, while *Brachionus* was most abundant at 18 °C (Bērzinš & Pejler, 1989). Additionally, an experimental study suggested that *Synchaeta* uses its resources most efficiently at low temperatures: Stelzer (1998) found that the TFL of *Synchaeta* increased almost 10-fold from 12 to 24 °C (see Fig. 1). In contrast, the TFL of *Brachionus* showed only minor differences over the same temperature range, being lowest at 16–20 °C and slightly elevated at 12 and 24 °C. As the isoclines connecting the TFLs at the different temperatures intersected at 15 °C, it was predicted that *Synchaeta* should outcompete *Brachionus* at temperatures below 15 °C while *Brachionus* should be the superior competitor above 15 °C, provided that both species compete for the same food resource (Stelzer, 1998).

The aim of this paper was to test these predictions in competition experiments at two temperatures. The hypotheses were: (i) *Synchaeta* will competitively displace *Brachionus* at 12 °C and (ii) *Brachionus* will competitively displace *Synchaeta* at 20 °C. In addition to the competition experiments, the temperature-dependence of feeding rates and starvation times were characterised for both rotifer species. This allowed the construction of a competition model to predict the time course of competitive exclusion. Resource concentrations were measured in each replicate to determine whether resource use and population dynamics of the two species were in accordance with theoretical predictions.

Methods

The *S. pectinata* clone was derived from an individual stem female that was isolated from Schöhsee (northern Germany). This clone has been characterised in detail in several previous publications and information on various life history parameters is available: population growth and TFLs at different temperatures (Stelzer, 1998), reproductive effort (Stelzer, 2001), and offspring investment in relation to temperature (Stelzer, 2002). The *B. calyciflorus* clone was obtained from K.O. Rothhaupt and is identical to the *B. calyciflorus* used in his work (e.g. Rothhaupt, 1990a). Information on population growth and TFLs was established in an earlier publication (Stelzer, 1998). Rotifers were raised on *Cryptomonas erosa* var. reflexa Skuja, which was obtained from J.J. Gilbert, New Hampshire. In all experiments the rotifers were cultured in ADaM medium (Klüttgen *et al.*, 1994), supplemented with 2.2 mg L^{-1} Na_2EDTA and WC medium (Guillard, 1975) at a volume ratio of 9 : 1 to improve the conditions for *Cryptomonas* (Kirk & Gilbert, 1990). *Cryptomonas erosa* was cultured in WC medium in semicontinuous culture (dilution rate = 0.25 day^{-1}) and was continuously illuminated (100 μmol quanta m^{-2} s^{-1}). The carbon content of *C. erosa* was 219 ± 8.7 pg $cell^{-1}$ and the average cell volume was 885 μm^3 (equivalent spherical diameter approximately 12 μm). Populations of rotifers were acclimated to the experimental temperatures for at least 2 weeks in 2-L Erlenmeyer flasks and at a food concentration of 3 mg C L^{-1}. Rotifers were cultured in continuous dim light (5 μmol quanta m^{-2} s^{-1}) to minimise algal growth in the cultures.

Feeding rates

The functional response of both rotifer species was determined at 12 and 20 °C. Rotifers were introduced into *Cryptomonas* suspensions at concentrations ranging from 0.1 to 1 mg C L^{-1}, which were provided in tissue culture plates (volume: 8 mL). Feeding trials lasted for 12 h and at least five replicates were run for each combination of temperature and food concentration. The experimental animals originated from populations that were placed into a food concentration of 0.328 mg C L^{-1} 1 day prior to the experiment. In order to avoid additional grazing by newly hatched offspring, only large individuals without eggs were

Table 1 Experimental design of the functional response experiments

Food concentration [*Cryptomonas* cells mL^{-1}]	*Brachionus* 12 °C	*Brachionus* 20 °C	*Synchaeta* 12 °C	*Synchaeta* 20 °C
500	8–14	4–7	26–28	16–20
1500	13–20	12–15	38–55	32–40
2500	41–45	11–20	92–100	45–50
3000	21–26	20–23	80–96	49–57
5000	70–82	14–27	150–185	55–60

Minimum and maximum number of individuals in the feeding trials (per 8 mL food suspension of each replicate).

selected from these cultures. The exact age of these individuals was not known but most of them were probably young adults as they produced eggs during the feeding trial. The numbers of rotifers in the feeding trials ranged from four animals (*Brachionus*, 20 °C, 0.1 mg L^{-1}) to 185 animals (*Synchaeta*, 12 °C, 1 mg L^{-1}). These numbers (see Table 1) had been estimated empirically from pilot experiments and were chosen to achieve a food depletion of approximately 10–50% during the incubation period. Control treatments without animals were run at all food concentrations and temperatures at the same time as the feeding trials. Algal concentrations were determined using an electronic particle counter (CASY, Schärfe GmbH, Reutlingen, Germany), set at a detection range of 8–15 μm equivalent spherical diameter. In an additional experiment, I determined the feeding rate for both rotifer species at a concentration of 0.328 mg C L^{-1} (1500 cells mL^{-1}), but at a wider range of temperatures (8, 10, 12, 16, 20 and 24 °C). Filtration rates F were calculated according to:

$$F = \frac{v(lnC_0 - lnC_t)}{t}$$

where v is the water volume per individual, C_0 is the mean concentration of algae in the control treatments, C_t is the concentration of algae at the end of the feeding trial, and t is the incubation time in days. The ingestion rate f was calculated according to the formula

$$f = F \cdot C_m$$

where C_m is the average food concentration during the experiment. I estimated C_m from initial and final algal densities in each replicate, assuming exponential decline during the experiment. Parameters of the functional response curves, such as the maximum feeding rate and the half-saturation constant, were estimated by fitting a curvilinear Michaelis–Menten model to the untransformed data of ingestion rates versus algal concentrations.

Starvation experiment

Starvation resistance at different temperatures (12, 16, 20 and 24 °C) was established for both rotifer species. Experiments were initiated with cohorts (hatching interval: 12–24 h, depending on temperature) of 24 young adults that had been cultured at a food concentration of 1500 cells mL^{-1} until they reached maturity (2–7 days, depending on temperature). Animals were then transferred into food-free medium and survival was recorded in 12 h-intervals until the last animal had died. Controls with fed animals were run parallel to these experiments. Almost all individuals in these controls survived until the end, indicating that the mortality in the food-free treatments was caused by starvation rather than old age. In *Brachionus*, only the temperatures 12–20 °C were tested, as the population that was acclimated to 24 °C inexplicably went extinct shortly before the experiment. Survival curves of both species were compared for each temperature separately using Gehan's Wilcoxon test for pairwise comparisons (Software: Statistica®, StatSoft Inc.).

Simulation model

Data from the functional response curves as well as data from the numerical response were used to parameterise a mathematical model of resource competition consisting of three differential equations representing the two consumers and their common resource:

$$\frac{dN_1}{N_1 dt} = \frac{r_{\mathrm{max}1}(S - S_{01})}{(K_{s1} + S - S_{01})} - D_{\mathrm{pred}}$$

$$\frac{dN_2}{N_2 dt} = \frac{r_{\mathrm{max}2}(S - S_{02})}{(K_{s2} + S - S_{02})} - D_{\mathrm{pred}}$$

$$\frac{dS}{dt} = D_{\mathrm{prey}}(S_v - S) - \left(\frac{N_1 \cdot f_{\mathrm{max}1} \cdot S}{K_{F1} + S}\right) - \left(\frac{N_2 \cdot f_{\mathrm{max}2} \cdot S}{K_{F2} + S}\right)$$

where N are the concentrations of the rotifers (*Brachionus*, *Synchaeta*), S is the concentration of the resource (*Cryptomonas*), $r_{\mathrm{max}}$is the maximum growth rate of the rotifers, K_s is the half-saturation constant of the numerical response, S_0 is the TFL for zero population growth, D_{pred} is the dilution rate applied to the rotifers, $f_{\max}$ is the maximum ingestion rate of the rotifers, K_F is the half-saturation constant of the functional response, D_{prey} is the dilution rate applied to the algae, and S_v is the concentration of the algae in the food supply. Note that algal concentrations in this model can only increase because of inflow from the food supply and that intrinsic algal growth is neglected. The model was parameterised using estimates of feeding rates from this study and estimates of population growth from an earlier study (Stelzer, 1998). The various parameter estimates are displayed in Table 2. Numerical simulations were carried out using initial densities of 1 individual mL^{-1} for each species and for an algal concentration of 0.328 mg C L^{-1} (1500 cells mL^{-1}) as food supply (S_v). The model was implemented in Matlab® (The MathWorks Inc.) and iterative calculations were run in time steps of 0.1 day.

Competition experiments

Two sets of competition experiments were run to test the predictions about competitive dominance between *S. pectinata* and *B. calyciflorus* at the different temperatures. In the first experiment, the animal populations were cultured in six-well tissue culture plates (8 mL volume). The *Cryptomonas* concentration in the supplied medium was 0.328 mg C L^{-1} (1500 cells mL^{-1}). Initial densities of the animals were 1 individual mL^{-1}. The experiment was initiated with a mixture of juveniles and adults (ratio 50 : 50) taken from a culture with a food concentration of 0.328 mg C L^{-1}. At intervals of 48 h all rotifers and their eggs were counted and transferred into a new vessel with fresh food suspension. The experiment was run at two temperatures (12 and 20 °C) and two competition regimes (alone, with competitor). Three replicates were performed of each treatment.

The main difference in the second competition experiment was the time interval at which the *Cryptomonas* solution was supplied. In this case, one-third of the culture medium was replaced by fresh food suspension every 8 h, resulting in a closer approximation to a continuous food supply. Rotifers were cultured in glass bottles (1100 mL volume) placed on plankton wheels, rotating every 15 min at 1 rpm for 2 min. The inoculation of each of these cultures was carried out with a mixture of 1100 juveniles and adults, in a similar manner to the first competition experiment. At intervals of 8 h, one-third of the medium was replaced by fresh food suspension (1500 cells mL^{-1}) by filtration through 30-μm gauze. Abundance of the two rotifer species and egg ratios were determined from daily samples of 50 mL volume. Egg ratio was defined as the number of eggs divided by the number of females in a sample. These were the only occasions when rotifers were harvested, resulting in a dilution rate of approximately 0.05 day^{-1}. This experiment was run only at 12 °C, as, given the strong competitive dominance of *Brachionus* in the first experiment, the question shifted towards whether *Synchaeta* could outcompete *Brachionus* at the lower temperature. Furthermore, for logistic reasons parallel trials at 20 °C were not possible. The experiment had three treatments (*Synchaeta* alone, *Brachionus* alone, both species together) and three replicates per treatment. Residual algae

Table 2 Parameters used in the competition model

	12 °C		20 °C	
	Synchaeta	*Brachionus*	*Synchaeta*	*Brachionus*
Numerical Response				
$r_{\max}$ [day^{-1}]	0.248	0.373	0.559	0.854
K_s [mg C L^{-1}]	0.084	0.297	0.354	0.208
S_0 [mg C L^{-1}]	0.074	0.183	0.275	0.132
Functional response				
$f_{\max}$ [μg C individual^{-1} day^{-1}]	0.059	0.14	0.122	0.307
K_F [mg L^{-1}]	0.122	0.092	0.396	0.072

Data based on Stelzer (1998) and the ingestion rates measured in this paper (see Fig. 3). $r_{\max}$, maximum growth rate; K_s, half-saturation constant of the numerical response; S_0, threshold food level for population growth; $f_{\max}$, maximum feeding rate; K_F, half-saturation constant of the functional response.

(i.e. the algal concentrations after an 8-h interval) were measured once per day using the electronic particle counter. This measurement provided an estimate of the minimal algal concentration each day. Maximal algal concentrations were calculated by taking into account the fresh food suspension that was replaced every 8 h.

Statistical analysis of the competition experiments was carried out for each temperature separately, using 2-way ANOVAs with time as a 'repeated-measures factor' to test if the presence of the competitor had a significant effect on population density of the focal species. In the second competition experiment, egg ratios of *S. pectinata* showed conspicuous fluctuations throughout the experiment. To test if these fluctuations had a periodic component, autocorrelation functions (ACFs) were calculated for each replicate (Software: Statistica®, time series analysis module). Prior to the autocorrelation analysis, data sets were ln-transformed and linear trends were removed, using the software's standard functions. Cycles in egg ratios were considered statistically significant if the ACF oscillated between positive and negative values and exceeded the 95% confidence limits at a time lag equal to the period length (Turchin & Taylor, 1992).

Results

Feeding rates of *B. calyciflorus* increased strongly with temperature, whereas those of *S. pectinata* stayed relatively constant at temperatures of 8–24 °C (Fig. 2). For instance, from 8 to 20 °C the ingestion rates of *Brachionus* at a food concentration of 0.328 mg C L^{-1} increased 6.2-fold, while in *Synchaeta* the increase was only 1.7-fold. This difference is reflected in a significant interaction of the factors 'species' × 'temperature' in the two-way ANOVA ($F_{6,55} = 37.46$, $P < 0.01$). Across the temperature range studied, feeding rates of *Brachionus* were generally higher than those of *Synchaeta* (up to 4.6-fold higher at 20 °C), although the difference between the two species diminished markedly at the lower temperatures. The higher feeding rates of *Brachionus* were also apparent under a wider range of food concentrations (see functional response curves, Fig. 3).

Starvation time in both rotifer species decreased with increasing temperature (Fig. 4). There was a strong difference in starvation resistance between the two rotifer species. At 12 °C, starvation times of *Brachionus* were almost 2.3 times longer than those of *Synchaeta*. At 20 °C this difference was weaker (1.5-fold). Mean survival at 20 °C was 2.3 days in *Brachionus* and 1.5 days in *Synchaeta*. The differences between species were statistically significant at all three temperatures, from 12 to 20 °C (Gehan's Wilcoxon test for pairwise comparisons, $P < 0.001$).

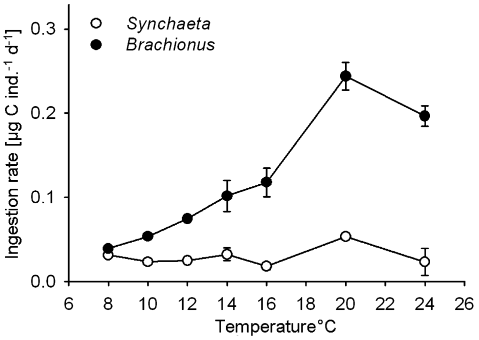

Fig. 2 Temperature-dependence of the ingestion rates of *Synchaeta* and *Brachionus* at a food concentration of 0.328 mg C L^{-1} (1500 cells mL^{-1} *Cryptomonas*). Means and standard errors.

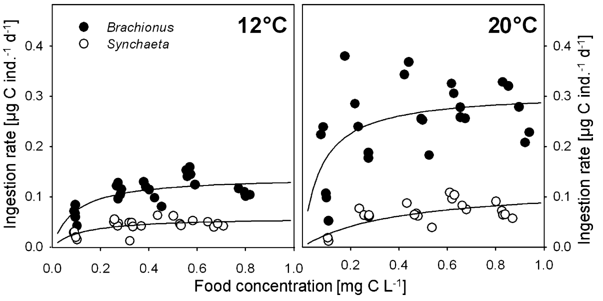

Fig. 3 Functional response curves of *Synchaeta* and *Brachionus* at 12 °C and 20 °C. Symbols: measured values; fitted lines: non-linear regression according to Michaelis–Menten models.

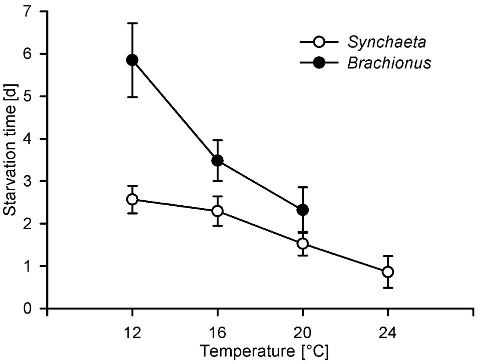

Fig. 4 Temperature-dependence of starvation times of adult *Synchaeta* and *Brachionus*. Mean and standard deviations.

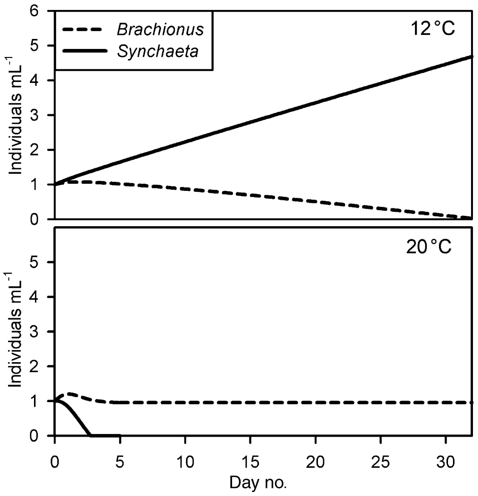

Fig. 5 Theoretical predictions of the time course of competition between *Synchaeta* and *Brachionus* at 12 and 20 °C. The competition model is based on the numerical response curves and functional response curves of the two competitors (parameter values, see Table 2).

The competition model was parameterised with the functional response data presented in Fig. 3 and data on growth rates of *Brachionus* and *Synchaeta* from an earlier publication (Stelzer, 1998). A summary of these parameter values is presented in Table 2. Initial densities of the competitors were set to 1 individual mL^{-1} and food concentration in the supply S_v was set to 0.328 mg C L^{-1}. Dilution rates were set to $D_{\text{prey}} = 1$ day^{-1} for the algae and $D_{\text{pred}} = 0.05$ day^{-1} for the rotifers, which mimics the experimental conditions in the second competition experiment. In the simulations *Synchaeta* was competitively displaced by *Brachionus* within 3 days at 20 °C (Fig. 5). In contrast, at 12 °C the simulation predicted *Synchaeta* to be the superior competitor. However, in this case the predicted time to competitive exclusion was much longer (30 days), although the *Brachionus* population was predicted to decline steadily after the fifth day.

In the first competition experiment *Brachionus* was the superior competitor at both temperatures (Fig. 6). *Synchaeta* was excluded after 4 days at 20 °C and after 16 days at 12 °C. In the controls without *Brachionus*, *Synchaeta* grew exponentially and built up large populations, up to 8 individuals mL^{-1} at 20 °C and 12 individuals mL^{-1} at 12 °C. The *Brachionus* populations grew more slowly in the presence of *Synchaeta* but this trend completely disappeared as soon as the *Synchaeta* populations died out. Afterwards the *Brachionus* populations in the competition treatment were not distinguishable from those in the single species treatments. Measurements of residual algae after each transfer interval showed that in the competition treatments algal concentrations fell below the detection limits of the electronic particle counter after only 2 days at 20 °C and after 6 days at 12 °C (data not shown).

The second competition experiment was run at 12 °C and was aimed to test the hypothesis that *Synchaeta* could outcompete *Brachionus* if they are provided with a feeding regime approximating a continuous food input (every 8 h). In all three replicates both competitors coexisted for 32 days and there

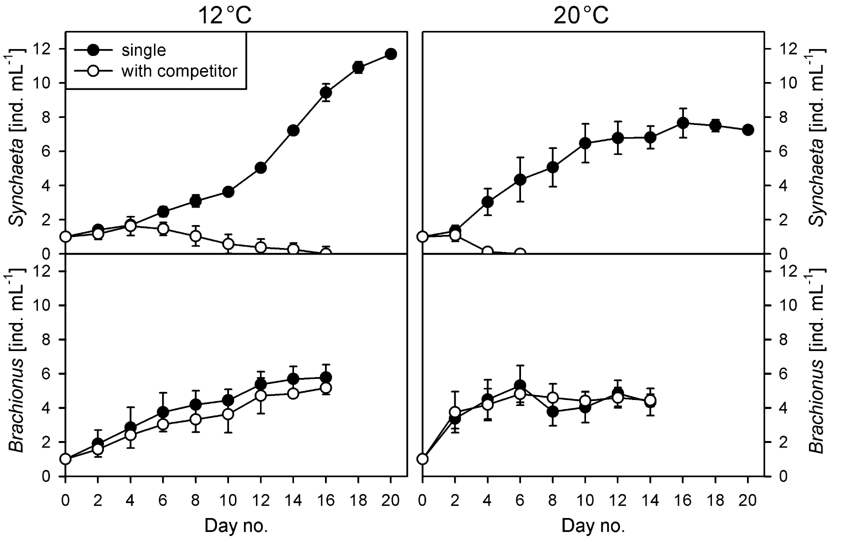

Fig. 6 Results of the first competition experiment. Time courses of abundances of *Synchaeta* and *Brachionus* in populations with and without competitors. Mean and standard deviations.

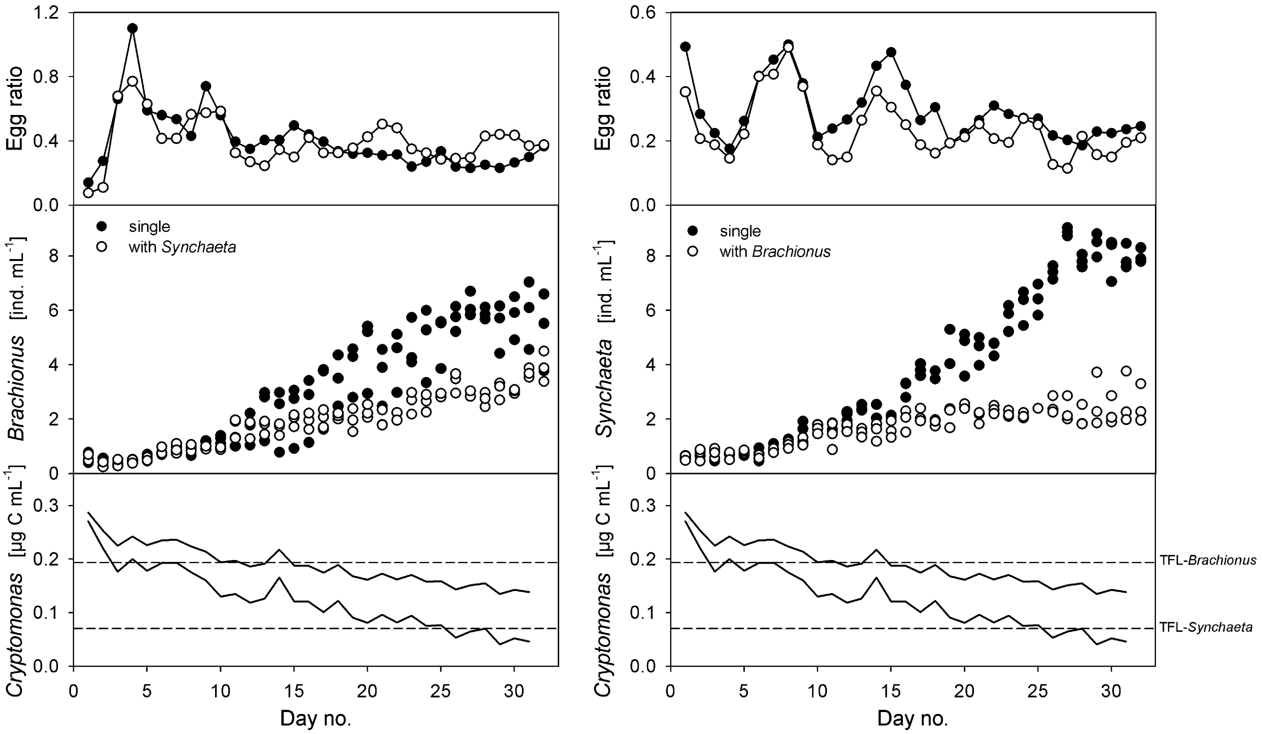

Fig. 7 Results of the second competition experiment. Dynamics of the *Brachionus* (left) and *Synchaeta* populations (right). Top: egg ratios; middle: rotifer abundances; bottom: maximal and minimal food concentrations in the competition treatments (lines), threshold food levels of the two rotifer species (dashed lines).

was no indication of competitive exclusion in either species (Fig. 7). However, both rotifer species were negatively affected by the presence of their competitor, as they build up smaller populations in mixed species cultures versus single species cultures (see Fig. 7; Table 3). During the 6 days of the experiment, when population size remained relatively stable in both species, *Synchaeta* populations were reduced on average by 70% in the presence of *Brachionus*, while *Brachionus* populations were reduced on average by 43% in the presence of *Synchaeta*. Concentrations of the food algae *Cryptomonas* fluctuated during the experiment, but these fluctuations took place over a relatively small range as can be seen from the maximal and minimal food concentrations in Fig. 7 (bottom graph). *Cryptomonas* concentrations declined steadily until they approached the TFL of *Syncheta*. After day 15, they were permanently below the TFL of *Brachionus*. Interestingly the *Brachionus* population was not decreasing at this time. In fact, it slightly increased in the second half of the experiment. In the control populations without *Synchaeta*, *Brachionus* also continued to grow after the *Cryptomonas* concentrations had already fallen below the TFL (Fig. 8). In contrast, the control populations of *Synchaeta* showed the expected pattern of population stagnation as *Cryptomonas* concentrations approached the TFL (Fig. 8).

Table 3 Results of the repeated-measures ANOVAS on rotifer abundances in the second competition experiment

Dependent variable	Factor	d.f.	*F*-value	*P*-value
Brachionus [individual mL^{-1}]	Syn	1,4	23.12	<0.01
	Time	31,124	43.41	<0.001
	Syn × time	31,124	6.04	<0.001
Synchaeta [individual mL^{-1}]	Brach	1,4	216.49	<0.001
	Time	31,124	161.7	<0.001
	Brach × time	31,124	68.89	<0.001

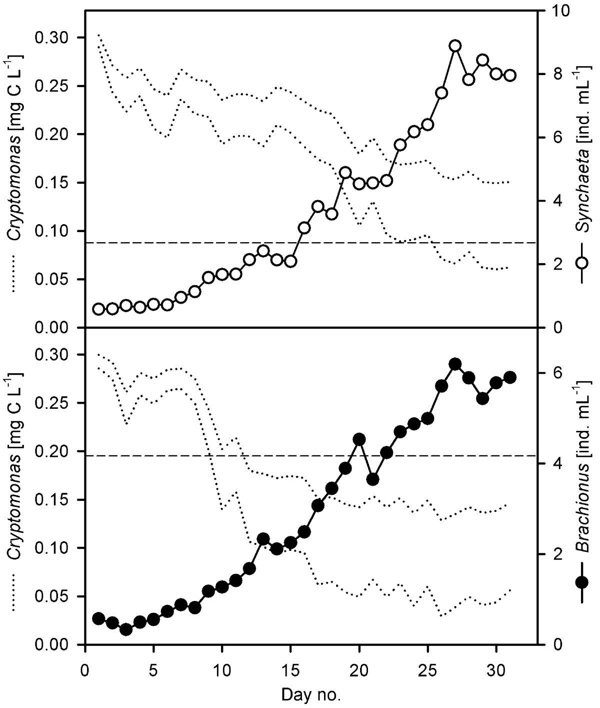

Fig. 8 Second competition experiment: dynamics in the single treatments (without competitors). Population dynamics of *Brachionus* and *Synchaeta* and concentrations of *Cryptomonas erosa*. Mean abundances (circles) and maximal/minimal food concentrations (dotted lines).

Egg ratios showed large fluctuations as well as an overall decline during the experiment (Fig. 7). Especially in *Synchaeta* these fluctuations in egg ratios seemed to have a periodic component (see Fig. 7, top right). Autocorrelation analysis showed that these cycles were significant in at least four replicates (data not shown), with the period of one cycle being 6–9 days. In the two remaining replicates the same trend was apparent but it was not statistically significant. In *Brachionus* there were no strong hints of a cyclic pattern in egg ratios. Only one replicate exhibited significant cycles of the ACF, with the remaining replicates showing no trends for periodicity.

Discussion

The main goal of this paper was to test the hypothesis that competitive abilities and the outcome of resource competition can reverse along a temperature gradient. Previous studies suggested that this should be the case when the two rotifers *S. pectinata* and *B. calyciflorus* compete for the food algae *C. erosa* at different temperatures (Stelzer, 1998). At low temperatures (12 °C) the TFL of *Synchaeta* was significantly lower than that of *Brachionus* and this situation was reversed at 20 °C. According to resource competition theory *Synchaeta* should displace *Brachionus* at 12 °C and *Brachionus* should displace *Synchaeta* at 20 °C. The results of this paper did not confirm this hypothesis. In the first competition experiment *Brachionus* displaced *Synchaeta* at both 12 and 20 °C. And in the second experiment, which was conducted at 12 °C, *Synchaeta* did not displace *Brachionus*. The species coexisted for more than 1 month and, although the abundances of both species were negatively affected by the presence of their competitor, there was no indication that *Synchaeta* would become dominant. The weak competitive performance of *Synchaeta* at 12 °C may require different explanations in the two competition experiments and will be discussed separately below.

In the first competition experiment, resources were supplied in a discontinuous manner. Every second day all animals were transferred into a new vessel containing food suspension at a concentration of 0.328 mg C L^{-1}. This concentration is well above the TFL of both species, but because of population growth and grazing it was reduced within a short time after the transfers. The discontinuous food supply probably favoured *Brachionus* for two reasons. First, *Brachionus* had higher feeding rates than *Synchaeta* and could therefore ingest more food within a short time. Second, *Brachionus* had a higher starvation tolerance and was better able to persist through the time periods of extremely low food abundance, which preceded each transfer interval in the later phase of the experiment. The reason for the difference in starvation tolerance probably lies in the contrasting reproductive strategies of the two species in response to food deprivation: *Brachionus* reduces its reproductive output and lowers its metabolism to survive periods of low food while *Synchaeta* actually increases reproduction at the expense of adult survival (Kirk, 1997; Stelzer, 2001, 2005). Fluctuating resource abundance can lead to competitive outcomes that deviate greatly from those predicted by the TFLs if the competitors show differential resistance to starvation (MacIsaac & Gilbert, 1991; Kirk, 2002). These mechanisms may have been responsible for the strong dominance of *Brachionus* in the first competition experiment.

Interestingly, many studies of resource competition have used an experimental protocol that avoids complications arising from starvation conditions. In such studies relatively high dilution rates of 0.2–0.7 day^{-1} were applied to the rotifer populations (Rothhaupt, 1988; Ciros-Perez *et al.*, 2001). An argument often made is that this mimics the natural mortality imposed by predators. However, as a result, the food concentrations that are necessary to maintain population equilibrium are much higher than the TFL. Accordingly, the inferior competitors are in a physiological state that cannot be termed 'starvation', even at times when they are being competitively displaced. Rather, they are excluded from the system because they do not grow fast enough to avoid the washout because of dilution. In contrast, the dilution rates of the rotifers in this study were much lower (0–0.05 day^{-1}), so the animals were closer to their actual TFL. Thus their population dynamics may have been determined mainly by their physiological response to starvation. This suggests that estimates of population growth rate (such as those obtained from a curve fit using the Monod-model) may not be accurate enough to predict long-term population dynamics in situations where resource concentrations are close to the TFL.

In the second competition experiment *C. erosa* was supplied at very short time intervals (8 h), which closely mimicked a continuous supply of food. Thus, resource fluctuations and differential tolerance to starvation cannot serve as explanations in this experiment. Measurements of the residual *Cryptomonas* concentrations indicated that resource concentrations fluctuated between feeding bouts, but within limits that were smaller than the difference between the TFLs of the two rotifer species. This means that from the middle of the experiment (approximately day 15) food concentrations were permanently below the TFL of *Brachionus*. However, the *Brachionus* population did not decrease as predicted from resource competition theory. In both the 'single species treatment' and in the 'competition treatment', *Brachionus* populations were stable even when the concentrations of *Cryptomonas* were far below the TFL. In contrast *Synchaeta* showed exactly the expected dynamics: populations grew exponentially until the *Cryptomonas* concentrations approached its TFL and then reached a stable equilibrium (Fig. 8). A possible explanation for the unusual behaviour of *Brachionus* is that they also fed on detritus and associated bacteria, which were inevitably accumulating during the later phase of the experiment. *Brachionus* is a generalistic consumer, feeding on algae of a wide range of sizes and it can even grow on bacteria (Starkweather, Gilbert & Frost, 1979). In contrast, *Synchaeta* is more specialised and can not ingest bacteria (Gilbert & Bogdan, 1984). *Brachionus* may thus have supplemented its diet with this additional food source. Such effects were probably absent in the experiments where the numerical response of *Brachionus* was established, as the culture medium was exchanged completely each day (Stelzer, 1998). Thus, in the second competition experiment there may have been resource partitioning that was not taken into account by the competition model. Several studies have demonstrated that resource partitioning can facilitate coexistence among consumers (Rothhaupt, 1988; Ciros-Perez *et al.*, 2001). A methodological improvement of the second competition experiment would be monoxenic cultures, involving only the rotifers and their food algae *Cryptomonas*, but without bacterial contaminants. Such monoxenic cultures have been described for rotifers (e.g. Gilbert, 1970), but are extremely difficult to maintain over long periods.

It is theoretically possible that the TFL of *Brachionus* at 12 °C was overestimated in the earlier study (Stelzer, 1998) and that this explains the good performance and the below-TFL population growth. Comparisons with literature data are restricted to 20 °C; however these do not indicate that the TFLs were overestimated. In fact, the value for the TFL of *Brachionus* at 20 °C of 0.13 mg C L^{-1} was even lower than that estimated by Rothhaupt (1990a) for a similarly sized algae, *C. sphaeroides* (TFL: 0.19 mg C L^{-1}). Stemberger & Gilbert (1985) obtained a similar value for *B. calyciflorus* at 19 °C (TFL: 0.38 mg L^{-1} dry weight of *C. erosa*). Also the TFL estimate for *Synchaeta* at 20 °C of 0.28 mg C L^{-1} comes very close to the 0.5 mg dry weight L^{-1} obtained by Stemberger & Gilbert (1985) assuming approximately 50% carbon content in the dried algal biomass. Thus, it seems rather unlikely that the unexpectedly good performance of *Brachionus* at low food concentrations was because of an overestimation of its TFL.

Data on growth and feeding suggest that *S. pectinata*, or at least the clone studied, is physiologically adapted to low temperatures. The *Synchaeta* clone can maintain positive population growth rates at temperatures as low as 4 °C (Stelzer, 2002). Data on growth rates of the *Brachionus* clone at such low temperatures are not available, but Halbach (1970) estimated 10 °C as the lowest temperature for population development in *B. calyciflorus*. If population growth rates are compared for different temperatures, *Synchaeta* reached 65% of its maximum growth rate at 12 °C, whereas *Brachionus* reached only 24% at the same temperature (Stelzer, 1998). Similarly, the relationship of ingestion rates versus temperature indicates that *Synchaeta* was feeding at close to maximum rates at 8 °C whereas the feeding rates of *Brachionus* showed a strong increase from 8 to 20 °C (Fig. 2). Similar indications of temperature adaptation were found by Gilbert & Bogdan (1984), who measured filtration rates of various rotifers *in situ*. They found monotonically increasing filtration rates with temperature in *Keratella cochlearis* Gosse and two *Polyarthra* species. Additionally they showed that the filtration rates of the cold-stenothermal *Polyarthra dolichoptera* Idelson at 7 °C were already as high as those of the eurythermal *Polyarthra vulgaris* Carlin at 20 °C (Gilbert & Bogdan, 1984).

The relationship between TFL and temperature may be an indicator of the temperatures to which an organism is adapted. In resource limited environments there is selection for very efficient use of food. Hence in a species that often faces resource limitation, the zones with the lowest TFLs may be a better indicator of the optimal temperature range than reaction norms that display growth rate versus temperature at high food concentrations. Several studies have demonstrated that rotifers, especially *S. pectinata*, are often food limited in their natural environment (Gonzalez & Frost, 1992; Merriman & Kirk, 2000; Stelzer, 2001). TFLs are expected to increase at higher temperatures if the assimilation rate has a temperature optimum but the rate of metabolic losses increase steadily with temperature (Lampert, 1977). The strong increase of TFL in *Synchaeta* may be caused by such mechanisms (Fig. 1). Over a very wide range of temperatures, optimum curves (U-shaped curves) are expected, in which the TFL stays constantly low over a preferred range of temperatures and only increases at the extremes. Such reaction norms have been described in cladocerans (Achenbach & Lampert, 1997) and a ciliate (Weisse *et al*., 2002).

An unexpected observation during the second competition experiment was the occurrence of periodic oscillations in the egg ratios of *Synchaeta*. It is unlikely that the egg ratio oscillations were triggered by fluctuations in resource abundance. First, resource concentrations (estimated as residual algae in the experimental vessels) did not show any cyclic fluctuations at all, but declined monotonically throughout the experiment. Second, the tendency for oscillations in egg ratios seemed to be much more pronounced in *Synchaeta* than in *Brachionus*, although both rotifers shared the same resource environment in the replicates of the competition treatment. The egg ratio oscillations were probably caused by the specific fecundity pattern of *Synchaeta*. Life table analysis of *S. pectinata* shows that production of offspring is maximal in the first adult age classes and then gradually decreases, displaying a 'triangular' fecundity pattern (Stelzer, 2001). In contrast, offspring production in *Brachionus* seems to be constantly high across the adult age classes, forming a 'rectangular' fecundity pattern (Halbach, 1970). This difference in fecundity schedule may be crucial, as a concentration of reproduction in a few age classes can slow down the convergence to a stable age structure (Caswell, 1989). A theoretical example of such cyclic age structure fluctuations is displayed in Fig. 9. Two hypothetical organisms are assumed whose life cycles are represented by the two Leslie matrices in Fig. 9. In the first organism, reproduction is concentrated in the first adult age class and then gradually declines (triangular fecundity pattern) whereas in the second organism reproduction is constant across all adult age classes (rectangular fecundity pattern). Both organisms have identical survival schedules and the same overall population growth rate ($r = 0.092$). The projections of their Leslie matrices reveals that both organisms exhibit dampened oscillations in age structure, represented by the ratio between the youngest age class and the sum of all remaining age classes (=egg ratio). In the organism with the triangular fecundity pattern these oscillations are more persistent than in the organism with the rectangular fecundity pattern, which shows one cycle with large amplitude at the beginning and then quickly converges to a stable egg ratio. Similar mechanisms may have caused the differential tendency of *Synchaeta* and *Brachionus* to form periodic cycles in egg ratios (Fig. 7).

In conclusion, the results of this study suggest that several mechanisms may cause deviations in the outcome of competition, as opposed to that predicted by the TFL. Resource fluctuations and differential

Triangular

$$\begin{bmatrix}5\\5\\5\\5\\5\end{bmatrix}\times\begin{bmatrix}0&0&1&0.4&0.1\\1&0&0&0&0\\0&1&0&0&0\\0&0&0.8&0&0\\0&0&0&0.6&0\end{bmatrix}$$

Rectangular

$$\begin{bmatrix}5\\5\\5\\5\\5\end{bmatrix}\times\begin{bmatrix}0&0&0.66&0.66&0.66\\1&0&0&0&0\\0&1&0&0&0\\0&0&0.8&0&0\\0&0&0&0.6&0\end{bmatrix}$$

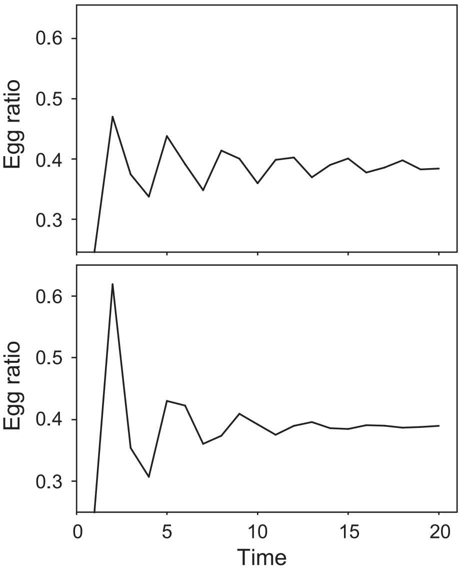

Fig. 9 Leslie matrices and periodic cycles of the egg ratios in two hypothetical organisms. Top: Organism with triangular fecundity pattern, showing fecundities of 1, 0.4 and 0.1 in the adult age classes. Bottom: Organism with rectangular fecundity pattern, showing fecundities of 0.66 in all adult age classes. The projections on the right show the temporal dynamics of the egg ratio for each Leslie matrix. Egg ratio is defined as the ratio between the abundance of the first age class divided by the sum of abundances in all remaining age classes. Starting condition: five individuals per age class.

tolerance of starvation may even reverse the outcome of competition. Unaccounted resource partitioning, such as in the second experiment, may cause additional deviations from theoretical predictions. It may be argued that such deviations could have been avoided by a finer tuning of the experimental conditions. However, resource fluctuations and complex food situations actually occur in most natural systems and are thus of ecological relevance. Finally, demographic effects, such as egg ratio oscillations represent another deviation from an assumption of resource competition theory, where populations are assumed to be in their stable age distribution. It remains to be investigated whether such deviations can actually affect the outcome of resource competition. Interestingly there is evidence from *Daphnia pulex* showing that temporal fluctuations in age structure can have strong impact on population dynamics and competition among clones (Nelson, McCauley & Wrona, 2005).

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